Gleevec inhibits β-amyloid production but not Notch cleavage

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Amyloid-β (Aβ) peptides, consisting mainly of 40 and 42 aa (Aβ40 and Aβ42, respectively), are metabolites of the amyloid precursor protein and are believed to be major pathological determinants of Alzheimer's disease. The proteolytic cleavages that form the Aβ N and C termini are catalyzed by β-secretase and γ-secretase, respectively. Here we demonstrate that γ-secretase generation of Aβ in an N2a cell-free system is ATP dependent. In addition, the Abl kinase inhibitor imatinib mesylate (Gleevec, or STI571), which targets the ATP-binding site of Abl and several other tyrosine kinases, potently reduces Aβ production in the N2a cell-free system and in intact N2a cells. Both STI571 and a related compound, inhibitor 2, also reduce Aβ production in rat primary neuronal cultures and *in vivo* in guinea pig brain. STI571 does not inhibit the γ-secretase-catalyzed S3 cleavage of Notch-1. Furthermore, production of Aβ and its inhibition by STI571 were demonstrated to occur to similar extents in both *Abl*$^{-/-}$ and WT mouse fibroblasts, indicating that the effect of STI571 on Aβ production does not involve Abl kinase. The efficacy of STI571 in reducing Aβ without affecting Notch-1 cleavage may prove useful as a basis for developing novel therapies for Alzheimer's disease.

Because of a large body of evidence implicating amyloid-β (Aβ) in Alzheimer's disease, therapies are being sought that reduce Aβ production and/or accumulation in brain (1, 2). Those efforts include attempts to suppress Aβ production by inhibiting either β-secretase (3) or γ-secretase activities. Several γ-secretase inhibitors have recently been described. They include transition state analogs that mimic the γ-secretase cleavage site on the immediate Aβ precursor, βCTF [the C-terminal fragment of β-amyloid precursor protein (βAPP)], and presumably compete with it for binding to the γ-secretase enzymatic site (4). Additionally, several nonsteroidal antiinflammatory drugs (NSAIDs) that are nonselective cyclooxygenase 1 and 2 inhibitors alter γ-secretase activity, resulting in reduced production of the highly amyloidogenic Aβ42 (5). Inhibition of glycogen synthase kinase 3 by LiCl has been reported to reduce Aβ (6, 7), perhaps through γ-secretase inhibition.

Here we have developed a strategy to inhibit production of Aβ. In a previous study, we reconstituted Aβ production in a cell-free system consisting of permeabilized mouse neuroblastoma (N2a) cells depleted of cytosol but containing intact membranes, and showed that optimal Aβ production requires incubation with an ATP-regenerating system (8). We hypothesized that this nucleotide requirement might offer insight into βAPP processing, and might also provide a target for suppressing Aβ production. Specifically, we have investigated the possibility of using inhibitors of the actions of ATP to affect Aβ production. One of these, STI571 (imatinib mesylate, or Gleevec), a selective tyrosine kinase inhibitor that binds to the ATP-binding sites of several tyrosine kinases, including Abl, ARG, platelet-derived growth factor receptor (PDGFR), and c-kit, is used to treat chronic myelogenous leukemia, a malignancy arising from activation of the Abl tyrosine kinase domain of the fusion protein, BCR-Abl (9, 10). Another compound, 6-(2,6-dichlorophenyl)-8-methyl-2-(3-methylsulfanylphenylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (inhibitor 2), has also been shown to inhibit Abl kinase by combining with its ATP-binding site (11–15).

Methods

N2a Cell Cultures and Incubation with STI571. Transfected N2a cells were grown as reported (16). Stock solutions of STI571 (10 mM; synthesized at the Organic Chemistry Core facility of Sloan–Kettering Institute) were made in DMSO (Sigma), or in water or normal saline for the mesylate salt (Gleevec, Novartis, Basel, Switzerland). The two formulations gave indistinguishable results. Inhibitor was rapidly mixed in culture medium and layered onto adherent cells grown in 60-mm tissue culture plates (Corning) and incubated for 3 or 16 h. Sixteen-hour incubation with inhibitors was carried out for cell cultures that were ≈100% confluent at the start of incubation to avoid the possibility that the inhibitors would prevent cell proliferation. In some experiments, overnight incubation with STI571 affected the level of expression of transgenic βAPP and other transfected genes, but not of endogenous βAPP. In all cases, Aβ levels were normalized to full-length βAPP for quantification.

Immunoprecipitation/Western Analysis. Immunodetection was carried out as reported (16) with the exception that Western blot analysis of Aβ involved incubating poly(vinylidene difluoride) (PVDF) membranes (Invitrogen Life Technologies) in PBS containing 0.2% glutaraldehyde (Sigma) for 45 min, after electrotransfer.

Preparation of Cell-Free Reconstitution System for Aβ Production. Cell-free reconstitution of Aβ production was carried out as reported (17) with modifications. Permeabilized cells were suspended in 20 mM Hepes, pH 6.0/2.5 mM $Mg(OAc)_2$/0.1 μM $CaCl_2$/110 mM KOAc and incubated at 37°C for 90 min in a final volume of 300 μl containing 40 μM mixed oligomycins (Sigma) to inhibit mitochondrial ATP generation (18). Additionally, either 15 μl of 40 mM ATP (final concentration, 2 mM) alone or an energy-regenerating mixture containing ATP, GTP (optional), creatine phosphate, creatine kinase (all from Roche Diagnostics), or 2 units of apyrase (grade VI, Sigma) without an energy-regenerating system, were included. No difference in Aβ production was seen between cells incubated with ATP alone or those incubated with the ATP-regenerating system. Levels of Aβ and βCTF were corrected for preincubation values.

Abbreviations: βAPP, β-amyloid precursor protein; sβAPP, soluble βAPP; Aβ, β-amyloid peptide; Aβ*n*, *n*-aa β-amyloid peptide; βCTF, C-terminal fragment of βAPP cleaved by β-secretase; SELDI, surface enhanced laser desorption/ionization; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide.

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SELDI (Surface Enhanced Laser Desorption/Ionization) Affinity Mass Spectrometry. Media were harvested from confluent N2a ΔE9/Swe cells exposed to 10 μM STI571 for 16 h. Antibody 6E10 (Signet Laboratories, Dedham, MA) or mouse anti-IgG antibody (ICN) was ligated to Ciphergen PS1 protein chips per the manufacturer's instructions, using the Ciphergen eight-well Bioprocessor Accessory (Ciphergen, Freemont, CA) (19, 20).

mNotchΔe Transfection and Notch-1 Cleavage Assays in N2a Cells. N2a cells transfected to stably overexpress βAPP Swedish were transiently transfected to overexpress mNotchΔE (truncated Notch-1, lacking most of the Notch extracellular domain with a C-terminal hemagglutinin tag) (21). Cultures were preincubated with STI571 or the γ-secretase inhibitor L-685,458 for 2 h, then pulse labeled for 30 min with [^{35}S]methionine/[^{35}S]cysteine Easy Tag Protein Labeling Mix (Perkin–Elmer) and chased for 2 h with inhibitors present. Media were immunoprecipitated with antibody 4G8 (Signet Laboratories), and Aβ was detected as described above. mNotchΔE was detected in cell lysates by immunoprecipitation using anti-myc antibody (Santa Cruz Biotechnology).

3T3 Fibroblast Cultures and Incubation with STI571. WT 3T3 fibroblasts (American Type Culture Collection) and 3T3 *Abl*$^{-/-}$ fibroblasts (generous gift of Y. E. Whang, University of California, Los Angeles) were grown in DMEM with 10% FBS and penicillin/streptomycin (50 U/50 μg/ml, respectively) (all from GIBCO) and incubated for 16 h with either 10 μM STI571 mesylate salt (Novartis) or normal saline, and 500 μCi (1 Ci = 37 GBq) of ^{35}S-labeling mixture followed by immunoprecipitation of media and cell lysates (antibody 4G8)/autoradiography as done for Aβ and βAPP previously.

Primary Neuronal Cultures. Cells were harvested from the cerebral cortices of embryonic day 18 (E18) embryos from timed-pregnant WT Sprague–Dawley rats (Charles River Breeding Laboratories) as reported (16). At day 3 of culture, STI571 (Sloan–Kettering Institute or Novartis) or inhibitor 2 (Organic Chemistry Core Facility, Sloan–Kettering Institute) was added and cells were incubated at 37°C for 16 h. Neurons were then incubated for an additional 4 h with 500 μCi of ^{35}S-labeling mixture in the presence of inhibitors. Media and cell lysates were immunoprecipitated with antibody 4G8 to detect Aβ and full-length βAPP or with antibody A4 to detect total soluble βAPP (sβAPP). For the time-course experiments, cells were exposed to 5 μM Gleevec (Novartis) or 1 μM inhibitor 2 (in DMSO) for 1–24 h; 500 μCi of ^{35}S-labeling mixture was added 4 h before the end of the incubation.

Intrathecal Delivery of Inhibitors. Osmotic minipumps (Alzet, Palo Alto, California) containing 200 μl of STI571 (mesylate salt, Novartis) in saline or 200 μl of inhibitor 2 in DMSO (loaded in a catheter) were implanted s.c. in 300- to 350-g 6-week-old WT male and female albino guinea pigs (Charles River Breeding Laboratories) with a catheter for subdural infusion at the base of the spinal cord, according to the manufacturer's instructions. Controls were treated with minipumps containing saline or DMSO. After 7 days of continuous delivery of inhibitors, animals were killed and the cortices were dissected out and homogenized in 25 mM Tris, pH 7.5/50 mM NaCl/1 mM DTT/5 mM EDTA/1 mM EGTA with a protease inhibitor mixture (Roche Diagnostics). Tissue homogenates were centrifuged at 100,000 × *g* for 1 h at 4°C. Pellets were further solubilized in 3% SDS in water containing 8 μl of 2-mercaptoethanol (Sigma) per ml and subjected to vortexing and heating at 95°C for 10 min. Solubilized cell pellets were sonicated and centrifuged at 100,000 × *g* for 15 min. Supernatants were diluted 10-fold in buffer consisting of 190 mM NaCl, 20 mM Tris·HCl (pH 8.8), 2 mM EDTA, and 2% Triton X-100 (Fisher Scientific). Samples were normalized to total protein and assayed for Aβ40/42 by sandwich ELISA according to the manufacturer's instructions (BioSource International, Camarillo, California).

Results

Cell-Free Reconstitution of Aβ Production. To identify the step in the βAPP processing pathway responsible for the stimulation of Aβ production by ATP, we compared accumulation of βAPP metabolites in a cell-free system consisting of N2a cells doubly transfected with two familial Alzheimer's disease-linked mutations: βAPP Swedish (22) and PS1, ΔE9 (23) (the doubly transfected cell line produces abundant Aβ). The presence of ATP resulted in a 3-fold increase in Aβ production compared with that observed in cells incubated with apyrase to hydrolyze endogenous ATP (24) (Fig. 1*A Left*). The presence of ATP also greatly reduced the accumulation of βCTF (Fig. 1*A Center*), consistent with an ATP-dependent stimulation of βCTF cleavage by γ-secretase (25, 26). Lactacystin, a proteosomal and lysosomal inhibitor (27), had no significant effect on βCTF levels in the presence or absence of ATP in this system (data not shown), supporting the idea that stimulation of γ-secretase activity by ATP was responsible for the reduction in βCTF accumulation rather than stimulation of degradation through a nonamyloidogenic pathway. Similar effects on β-amyloid and βCTF accumulation were observed whether ATP was used alone or in the presence of an energy-regenerating system containing creatine phosphate and creatine kinase, with or without GTP (data not shown). Taken together, these results indicate that, in permeabilized cells, optimal Aβ production requires γ-secretase activity that is stimulated by ATP, and that substantial β-secretase activity can occur in the absence of ATP.

To further investigate the effect of ATP on γ-secretase activity, a cell-free system was reconstituted from N2a cells that had been transfected with a human C99-expressing transgene (28). When this construct is used, an effect of ATP on Aβ production could result only from an action on γ-secretase, not on β-secretase. By using antibody 6E10, which recognizes only the human transgene product, we were able to eliminate any detection of endogenous mouse Aβ. In this system, Aβ levels were increased in the presence of ATP (Fig. 1*A Right*), further indicating an ATP requirement for optimal γ-secretase activity.

We next tested whether Gleevec (STI571), which by virtue of competing at ATP-binding sites (12) is a potent tyrosine kinase inhibitor, might affect Aβ production in permeabilized N2a 695 cells. Before permeabilization, intact cells were incubated overnight with 10 μM STI571. After permeabilization and continued incubation with STI571 at 37°C in the presence of ATP, Aβ production was greatly reduced compared with permeabilized cells that had not been exposed to STI571 (Fig. 1*B*). The inhibitory effect of STI571 was lost in the absence of added ATP.

Inhibition of Aβ Production by STI571 in Intact N2a Cells. We next tested whether STI571 might affect Aβ production in cultured N2a ΔE9/Swe cells. Incubation of cells for 16 h with 10 μM STI571 resulted in an ≈50% decrease in secreted (Fig. 2*A*) and cellular (data not shown) Aβ. Total βAPP and sβAPPα remained unchanged (Fig. 2*A*). A small (but not significant) increase in βCTF was observed, consistent with γ-secretase inhibition (Fig. 2*A*). Aβ secretion by N2a cells was reduced to a similar extent after 3 h, as compared with 16 h of incubation with STI571 (data not shown). Analysis of conditioned media from N2a cells treated with 10 μM STI571 for 16 h by SELDI mass spectrometry showed a proportional decline in Aβ1–38, Aβ1–40, and Aβ1–42 (Fig. 2 *B* and *C*).

To test the ability of STI571 to inhibit γ-secretase activity in intact cells, cultures of N2a cells that stably overexpress human C99 (the γ-secretase substrate) were incubated with 10 μM

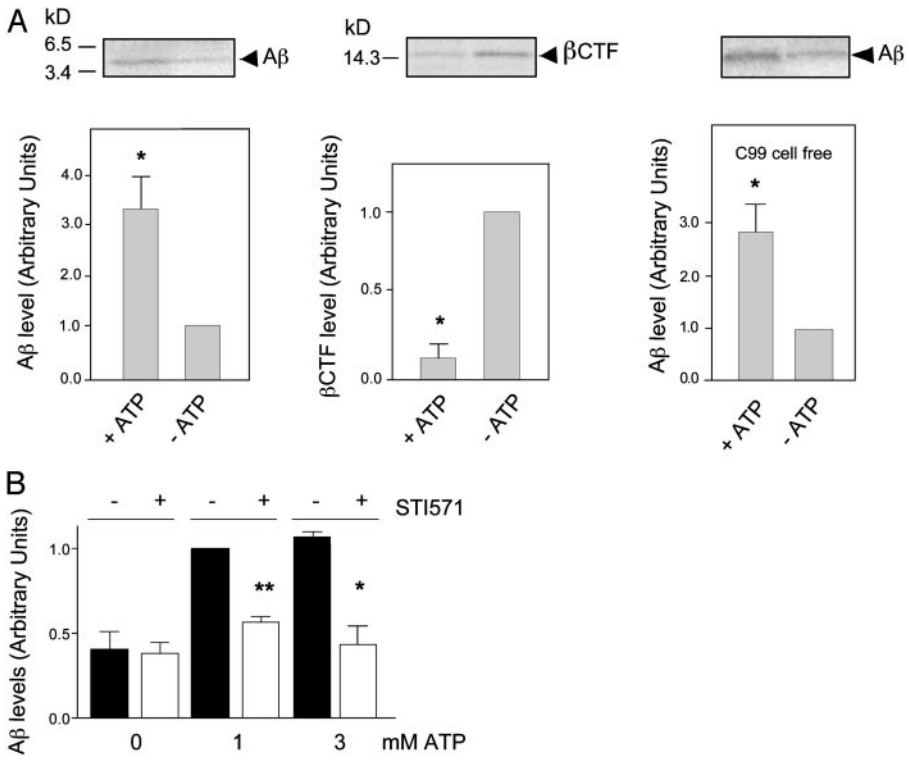

Fig. 1. γ-Secretase cleavage of βAPP is stimulated by ATP and inhibited by STI571 in an N2a cell-free system. (*A*) ATP dependence of Aβ and βCTF accumulation. N2a cells overexpressing either the human βAPP Swedish mutation plus the human PS1 mutation, ΔE9 (N2a ΔE9/Swe) (*Left* and *Center*), or human C99 (*Right*); Aβ production (*Left* and *Right*) and βCTF production (*Center*). $n = 3$, error bar represents SEM; *, $P < 0.01$, compared with no ATP. (*B*) Effect of STI571 on Aβ generation. N2a cells overexpressing human βAPP 695 were incubated with or without STI571 and 0, 1, or 3 mM ATP. $n = 3$, SEM; *, $P < 0.01$, **, $P < 0.001$, compared with absence of STI571.

STI571 for 4 h. This resulted in an ≈30% inhibition of secreted Aβ (Fig. 2*D*), less than that observed for N2a cells that overexpress full-length βAPP, nevertheless consistent with an action on γ-secretase activity. In N2a cell cultures incubated with 10 μM STI571 for 24 h, cell viability was unaffected as measured by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay (29) and trypan blue exclusion (data not shown), ruling out cytotoxicity as a cause of Aβ reduction. In fact, STI571 was shown, using the MTT assay, to produce a moderate protection of N2a cells against cytotoxicity induced by exposure to 100 mM glutamate for 24 h (unpublished data).

STI571 Does Not Inhibit Notch-1 Cleavage. Most γ-secretase inhibitors also inhibit cleavage of the γ-secretase substrate, Notch-1, a transcription factor that functions in neural development and in the adult immune system (30). In contrast, certain nonsteroidal antiinflammatory drugs (NSAIDs) that reduce Aβ42 levels do not inhibit Notch-1 cleavage (5). To determine whether STI571 inhibits Notch cleavage, we exposed N2a Swedish cells coexpressing N-terminally truncated Notch-1 (mNotchΔE) to various concentrations of STI571, as well as to the γ-secretase inhibitor L-685,458 (31). STI571 did not affect Notch cleavage at any concentration tested (Fig. 3), whereas concomitant Aβ production was reduced in a dose-dependent manner (Fig. 3*B*). In contrast, Notch cleavage was potently inhibited by L-685,458 at concentrations that produced a dose-dependent decrease in Aβ (Fig. 3).

STI571 Lowers Aβ in the Absence of Abl Kinase. The principal target of STI571 in chronic myelogenous leukemia therapy is believed to be the Abl kinase domain of the fusion protein BCR-Abl (32). To determine whether Abl kinase (in nonleukemic cells) is involved in the Aβ pathway that is inhibited by STI571, we compared Aβ production in Abl-knockout 3T3 fibroblasts ($Abl^{-/-}$) (33) and WT 3T3 fibroblasts in the absence and presence of STI571 (Fig. 4). The two cell lines secreted similar levels of Aβ (normalized to expression of endogenous βAPP; data not shown) and Aβ production was inhibited to a similar extent by STI571 in the two cell types, indicating that Aβ production and its inhibition by STI571 do not depend on Abl kinase. This result is consistent with the observation that inhibition of Aβ production requires higher concentrations of either STI571 or inhibitor 2 than does inhibition of Abl (IC_{50}: 3 μM vs. 40 nM and 300 nM vs. 2 nM, respectively) (12).

STI571 and Inhibitor 2 Inhibit Aβ Production in Rat Primary Neuronal Cultures. We next examined whether STI571 and a related compound, inhibitor 2 [originally identified as a Src inhibitor and subsequently found to inhibit Abl (11, 34)], might lower Aβ production in untransfected rat embryonic primary neuronal cultures. Both inhibitors caused an inhibition of Aβ production (Fig. 5 *A* and *B*). Inhibitor 2 had an IC_{50} ≈1/10 that of STI571. Whereas a maximal effect of STI571 on Aβ production in N2a cells occurred within 2–3 h, the effect on neurons required a longer period of time (Fig. 5*C*). No effect of STI571 or inhibitor 2 was observed on full-length βAPP. No inhibition of sβAPP was seen (Fig. 5*A*), arguing against a change in Aβ being secondary to an alteration in α-secretase activity. A cell viability assay that uses the metabolically sensitive dye MTT showed (as with N2a cells) that STI571 protected neurons against glutamate-induced toxicity (unpublished data).

STI571 and Inhibitor 2 Inhibit Aβ Production *in Vivo*. We next investigated whether STI571 and inhibitor 2 might inhibit Aβ

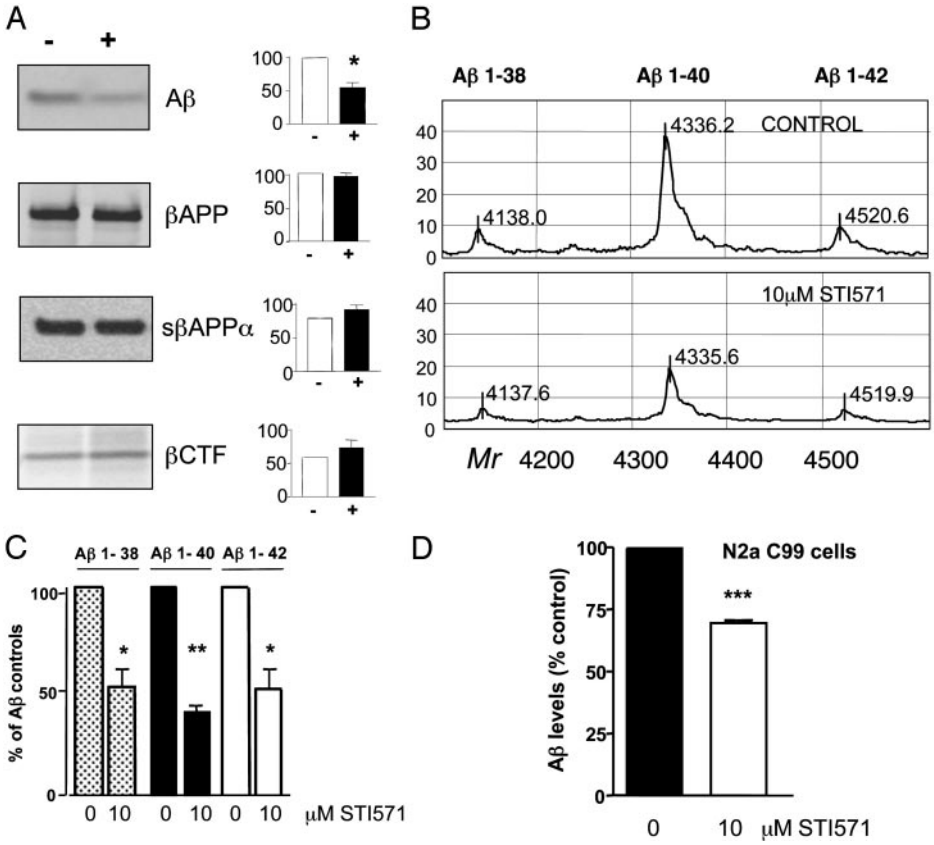

Fig. 2. Inhibition of Aβ production by STI571 in intact N2a cells. (*A*) Cultured N2a ΔE9/Swe cells were incubated with (+) or without (−) STI571. Secreted Aβ1–40/42, full-length βAPP, sβAPPα, and βCTF levels are compared. $n = 4$, SEM; *, $P < 0.01$. (*B* and *C*) SELDI mass spectrographic analysis comparing Aβ1–38, Aβ1–40, and Aβ1–42 secreted from N2a ΔE9/Swe cells incubated with or without STI571. (*B*) Typical mass spectra. (*C*) Quantification. $n = 3$, SEM; *, $P < 0.05$, **, $P < 0.01$. (*D*) Secreted Aβ1–40/42 levels produced by cultured N2a C99 cells incubated with or without STI571. $n = 3$, SEM; ***, $P < 0.005$.

production *in vivo*. We chose normal WT albino guinea pigs as a model, because their Aβ peptides are identical to human Aβ and can be readily detected by sandwich ELISA using anti-human Aβ40/42 antibodies (35). It has been shown that STI571 does not penetrate the blood–brain barrier efficiently (ref. 36; B. Druker, personal communication). We therefore delivered each inhibitor intrathecally over 7 days by means of implanted osmotic minipumps (37). STI571 (0.22 or 1.1 mg/kg per day) and inhibitor 2 (0.2 or 0.4 mg/kg per day) lowered brain Aβ40 by ≈50% (Fig. 6*A*). The same doses of these compounds caused similar decreases in Aβ42 levels, but these decreases were not statistically significant; a high variability in Aβ42 measurement was observed, probably attributable to the small amounts present or to nonspecific recognition of βCTF by the anti-Aβ42 antibody (38). βCTF levels increased significantly in treated animals (Fig. 6*B*) with no change in full-length βAPP levels (data not shown), consistent with an inhibitory action on γ-secretase (39). No obvious signs of behavioral or anatomic abnormalities were observed for any of the treated animals at the indicated doses. Higher doses for both inhibitors were no more effective than lower doses and in some cases less effective, possibly because of toxicity or because the drugs may have precipitated in the pumps or catheters and failed to be adequately delivered; precipitation occurred in stock solutions of inhibitor 2 greater than 20 mM stored at room temperature for >24 h.

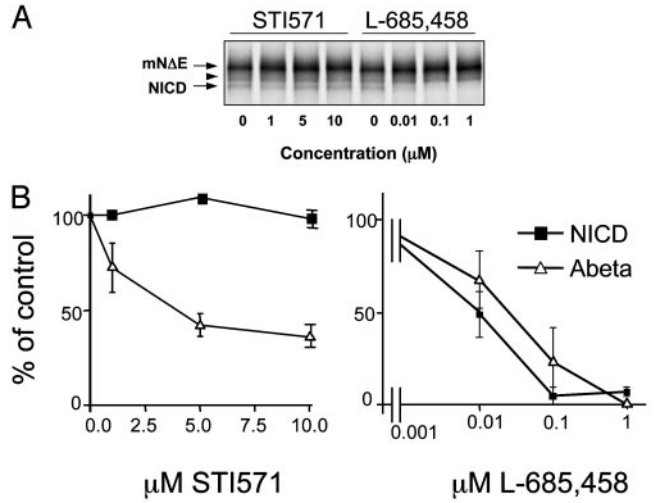

Fig. 3. Notch-1 S3 cleavage is inhibited by γ-secretase inhibitor L-685,458 but not by STI571. N2a βAPP Swedish cells overexpressing mNotchΔE were incubated with STI571 or L-685,458. (*A*) Upper band, full-length mNotch ΔE; lower band, γ-secretase cleavage product, NICD; middle band, cotranslational product unrelated to γ-secretase activity. (*B*) Comparison of effects of inhibitors on mNotchΔE cleavage and secreted Aβ1–40/42. $n = 3$.

Discussion

The present study indicated that STI571 caused a dose-dependent inhibition of γ-secretase cleavage of βAPP and of C99 but not of Notch-1 in mouse neuroblastoma (N2a) cells transfected with human βAPP. Additionally, STI571 and inhibitor 2 inhibited Aβ production from endogenous βAPP in rat embryonic primary neurons, demonstrating that the Aβ

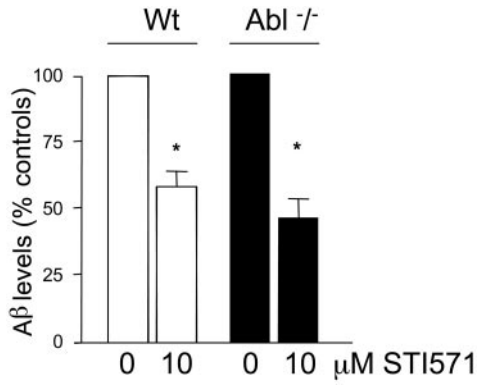

Fig. 4. Inhibition of secreted Aβ production by STI571 in cultures of WT and *Abl*$^{-/-}$ 3T3 fibroblasts. Values for each genotype are expressed as percent of secreted Aβ production in cultures not treated with STI571. $n = 3$, SEM; *, $P < 0.05$, compared with control.

lowering effect is not peculiar to cell lines overexpressing mutant human forms of βAPP. Moreover, intrathecal infusion of either STI571 or inhibitor 2 potently inhibited brain Aβ production in guinea pigs and, consistent with γ-secretase inhibition, raised βCTF levels.

The data indicate a differential effect of STI571 on βAPP processing vs. Notch-1 cleavage. Differences in the regulation of βAPP cleavage and Notch-1 cleavage have previously been observed (40, 41). In addition, γ-secretase cleavage of βAPP

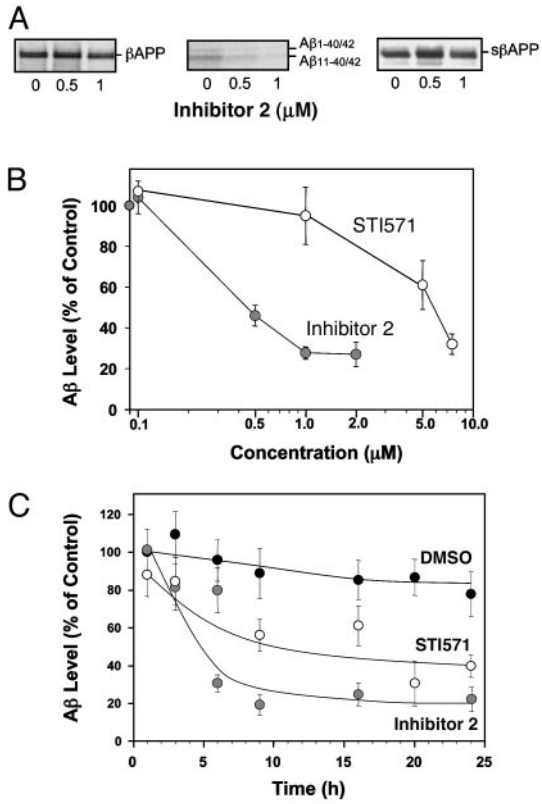

Fig. 5. Inhibition of Aβ secretion by STI571 and inhibitor 2 in cultured primary rat neurons. (*A*) Neurons were incubated with or without inhibitor 2. Full-length βAPP, Aβ1–40/42, Aβ11–40/42, and sβAPP are shown. (*B*) Dose–response curves for neurons exposed to various concentrations of inhibitor 2 or STI571 for 20 h. (*C*) Neurons were preincubated with 5 μM STI571, 1 μM inhibitor 2, or DMSO for the indicated times. $n = 4$.

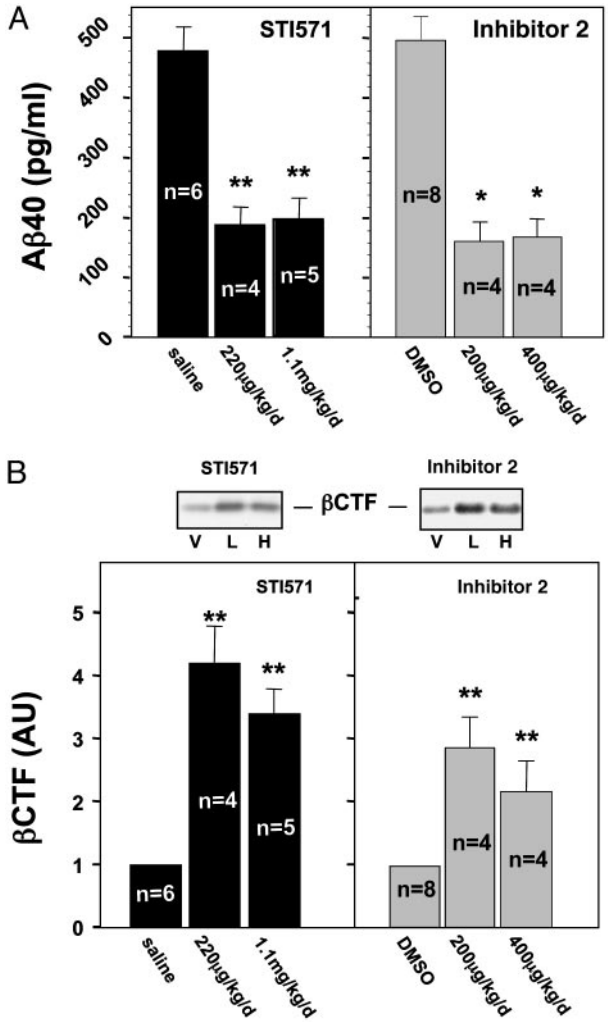

Fig. 6. Regulation of Aβ40 and βCTF accumulation in adult albino guinea pig brain by STI571 and inhibitor 2 . (*A*) Aβ40 levels in cortex after administration of STI571, inhibitor 2 , or vehicle (saline or DMSO, respectively). (*B Lower*) βCTF levels (in arbitrary units) in cortex after administration of STI571, inhibitor 2, or vehicle. *n* as shown, SEM; *, $P < 0.05$; **, $P < 0.001$. (*Upper*) βCTF Western blots. V, vehicle; L, lower dose; H, higher dose, as specified in bar graphs.

occurs in secretory compartments (8), whereas γ-secretase cleavage of Notch-1 occurs at or near the plasma membrane (42). Conceivably, STI571 and inhibitor 2 might act on a regulator of γ-secretase activity that functions preferentially in Aβ production.

Although the Abl kinase domain (of BCR-Abl) is an important target of Gleevec in chronic myelogenous leukemia, it does not appear that Abl kinase is required for Aβ production, or for Aβ inhibition by STI571 as indicated by the results comparing WT and *Abl*$^{-/-}$ 3T3 fibroblasts. Because STI571 and inhibitor 2 target several tyrosine protein kinases in addition to Abl, we cannot rule out the possibility that these inhibitors produce their effects on Aβ processing through inhibition of one of these other tyrosine kinases [e.g., ARG, platelet-derived growth factor receptor (PDGFR), Src, c-kit]. In support of this possibility, evidence using cell lines has just been reported, suggesting that PDGFR and Src kinase might play a role in Aβ production (43). Elucidation of the mechanism by which STI571 and inhibitor 2 disrupt γ-secretase cleavage of its βAPP substrate (but not of Notch) awaits further investigation. The mechanism of action of the inhibi-

tors could involve an effect on the localization of γ-secretase or of βAPP in a way that prevents interaction of the βAPP substrate with γ-secretase.

In conclusion, we have provided *in vitro* and *in vivo* evidence of another therapeutic approach to Alzheimer's disease, involving pharmacophores that direct binding to an ATP-binding site of a currently unidentified target protein. The safety of Gleevec, demonstrated by its successful application to chronic myelogenous leukemia and more recently to gastrointestinal stromal tumors, and its inability to inhibit Notch-1 cleavage by γ-secretase make this class of compounds attractive as potentially safe, Aβ-lowering drugs for the treatment of Alzheimer's disease. In the case of Gleevec and related drugs, the ability to achieve a high degree of penetration of the blood–brain barrier would be necessary to improve the likelihood of therapeutic benefit.

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